FEBS Letters 544 (2003) 4–10 FEBS 27286

Minireview

Microbial isoprenoid biosynthesis and human γδ T cell activation

Matthias Eberl*, Martin Hintz, Armin Reichenberg, Ann-Kristin Kollas, Jochen Wiesner, Hassan Jomaa

Biochemisches Institut, Justus-Liebig-Universität Giessen, Friedrichstr. 24, 35392 Giessen, Germany

Received 14 March 2003; revised 16 April 2003; accepted 17 April 2003

First published online 12 May 2003

Edited by Beat Imhof

Abstract Human Vγ9/Vδ2 T cells play a crucial role in the immune response to microbial pathogens, yet their unconventional reactivity towards non-peptide antigens has been enigmatic until recently. The break-through in identification of the specific activator was only possible due to recent success in a seemingly remote field: the elucidation of the reaction steps of the newly discovered 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway of isoprenoid biosynthesis that is utilised by many pathogenic bacteria. Unexpectedly, the intermediate of the MEP pathway, (*E*)-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate) (HMB-PP), turned out to be by far the most potent Vγ9/Vδ2 T cell activator known, with an EC_{50} of 0.1 nM.
© 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: GcpE; LytB; DOXP; MEP; HMB-PP; IPP

1. Introduction

In humans, γδ T cells constitute 0.5–15% of peripheral blood T cells and comprise a number of subpopulations characterised by the distinct repertoire of antigen receptors encoded by Vγ- and Vδ-gene segments. Specific activation and oligoclonal expansion of γδ T cells bearing a Vγ9/Vδ2 T cell receptor is observed after infection with a broad range of microbial pathogens, and considered crucial for the regulation of the immune response in diseases like tuberculosis or malaria. While the response of Vγ9/Vδ2 T cells depends on the expression of a functional Vγ9/Vδ2 T cell receptor on the surface, it does not require classical antigen presentation in the context of the major histocompatibility complex, as opposed to the conventional recognition of small antigenic peptides by αβ T cells. Also, the unique reactivity of Vγ9/Vδ2 T cells towards low molecular weight compounds (< 1 kDa) that are resistant against proteinase K treatment but susceptible to degradation by alkaline phosphatase has long puzzled immunologists, even more so as it seems to be restricted to humans and higher primates [1–4]. Strikingly, the most recent identification of the specific and naturally occurring activator of human Vγ9/Vδ2 T cells and the elucidation of the terminal reaction steps in a newly discovered, alternative pathway of isoprenoid biosynthesis went hand in hand.

2. The MEP pathway of isoprenoid biosynthesis

Isoprenoids are essentially involved in primary and secondary metabolism, and include such diverse molecules as sterols, dolichols, plastoquinones, ubiquinones, carotenoids, and the prenyl side chains of chlorophylls. However, despite their structural and functional variety, all isoprenoids derive from a common precursor, isopentenyl pyrophosphate (IPP), and its isomer, dimethylallyl pyrophosphate (DMAPP). Until very recently, IPP was thought to be synthesised exclusively via the so-called mevalonate pathway, with 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase as the key regulatory enzyme. The reaction steps of the mevalonate pathway of isoprenoid biosynthesis were first demonstrated in mammalian cells and yeast, but soon accepted to be universal in all organisms [5]. Yet unexpectedly, the existence of a second isoprenoid pathway was discovered in 1993 during isotope incorporation studies in a number of bacteria and plant species [6–9]; this pathway was later named after its key intermediate, the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. It has now become clear that in eukaryotes, archaebacteria, and certain eubacteria, biosynthesis of IPP and DMAPP proceeds entirely via the classical mevalonate pathway, while in many eubacteria and the plastids of algae and higher plants, IPP and DMAPP are supplied by the MEP pathway [10–12]. Also, apicomplexan protozoa like the malarial parasites harbour the MEP pathway in a plastid-like organelle, the apicoplast [13].

Pioneer work postulated the formation of 1-deoxy-D-xylulose 5-phosphate (DOXP) from pyruvate and glyceraldehyde 3-phosphate as the starting point of the alternative non-mevalonate pathway of isoprenoid biosynthesis, and accordingly identified the responsible enzyme, DOXP synthase (Dxs), by its homology to known transketolases [14,15]. Subsequent investigations could reveal that DOXP is then converted into MEP by DOXP reductoisomerase (Dxr, IspC) [16–18]. (It is worth stressing that apart from being involved in the MEP pathway, DOXP is also a precursor of thiamine and pyridoxal, thus making MEP the first true intermediate of the alter-

*Corresponding author. Fax: (49)-641-99 47499.
E-mail address: matthias.eberl@biochemie.med.uni-giessen.de (M. Eberl).

Abbreviations: DMAPP, dimethylallyl pyrophosphate; DOXP, 1-deoxy-D-xylulose 5-phosphate; Dxr, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; Dxs, 1-deoxy-D-xylulose 5-phosphate synthase; HMB-PP, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IPP, isopentenyl pyrophosphate; MEcPP, 2-*C*-methyl-D-erythritol 2,4-cyclopyrophosphate; MEP, 2-*C*-methyl-D-erythritol 4-phosphate

0014-5793/03/$22.00 © 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00483-6

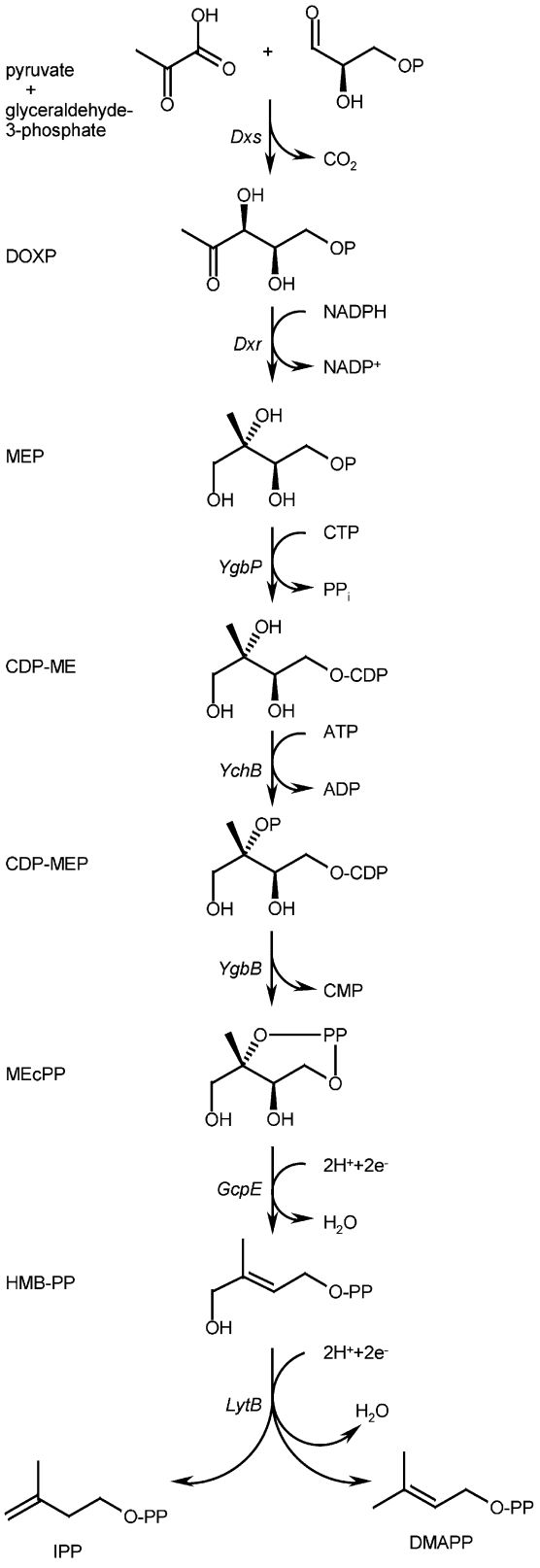

Fig. 1. The reaction steps of the MEP pathway of isoprenoid biosynthesis.

native pathway of isoprenoid biosynthesis, and justifying the now commonly accepted reference as to the MEP pathway.) Finally, the enzymes encoded by the genes *ygbP* (*ispD*), *ychB* (*ispE*), and *ygbB* (*ispF*) were shown to mediate the subsequent formation of 2-*C*-methyl-D-erythritol 2,4-cyclopyrophosphate (MEcPP) via three consecutive reaction steps [19–21] (Fig. 1).

Despite this rapid progress, the biochemical conversion of MEcPP into IPP and DMAPP has only now been understood. An additional role for the genes *gcpE* (*ispG*) and *lytB* (*ispH*) in IPP formation via the MEP pathway was demonstrated using knock-out techniques. *Escherichia coli* strains deficient in *gcpE* and *lytB*, respectively, that utilise exogenously provided mevalonate for IPP synthesis were created by complementation with plasmids expressing the heterologous enzymes of the mevalonate pathway. In consequence, the mutants were only viable when the culture medium was supplemented with mevalonate, thereby by-passing the defect in IPP formation, whereas under normal circumstances deficiency in either *gcpE* or *lytB* was lethal [22–24]. In other studies, addition of the alcohol analogues of IPP and DMAPP (3-methyl-3-buten-1-ol and 3-methyl-2-buten-1-ol, respectively) was necessary to support the growth of *lytB*-deficient mutants of *E. coli* and the cyanobacterium *Synechocystis* [25,26]. However, the enzymatic activity of the corresponding enzymes GcpE and LytB could not be resolved by these studies, and the gap between MEcPP and IPP still needed to be filled.

3. Identification of (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) as the natural γδ T cell activator

Although IPP was isolated from *Mycobacterium smegmatis* as the first 'natural ligand' described for Vγ9/Vδ2 T cells [27], it soon became clear that the amounts of IPP present in bacterial extracts do not reach the minimum required for inducing T cell activation [28]. Instead, the biosynthesis of IPP could be implicated in Vγ9/Vδ2 T cell reactivity. A comparison between extracts from bacteria possessing the classical mevalonate pathway of IPP synthesis, such as *Lactobacillus casei* and *Staphylococcus aureus*, and bacteria possessing the MEP pathway, such as *E. coli* and *Corynebacterium ammoniagenes*, revealed that only the latter are capable of stimulating Vγ9/Vδ2 T cells in vitro [28]. The final proof that compounds synthesised by the MEP pathway are indeed responsible for this phenomenon, came from genetically engineered *E. coli* strains, with essential enzymes of the MEP pathway being disrupted or deleted from the genome. Low molecular weight extracts prepared from Δ*dxr* and Δ*gcpE* mutants had a significantly reduced capacity to stimulate Vγ9/Vδ2 T cells, compared to the parent *E. coli* strain [29], thus proving the importance of the MEP pathway for biosynthesis of the γδ T cell activator. Addition of 2-*C*-methyl-D-erythritol to the bacteria broth by-passed the deficiency in *dxr* [30], and consequently restored the immunogenicity [29]. However, none of the known intermediates DOXP, MEP, CDP-ME (4-diphosphocytidyl-2-*C*-methyl-D-erythritol), CDP-MEP (4-diphosphocytidyl-2-*C*-methyl-D-erythritol 2-phosphate), and MEcPP exerted any T cell stimulatory activity [28,31–33].

In striking contrast to the case of Δ*dxr* and Δ*gcpE* mutants, extracts from Δ*lytB* bacteria were highly immunogenic, and the Vγ9/Vδ2 T cell-stimulating molecule accumulated by a factor of approx. 150, compared to wild-type *E. coli* [34]. The compound accounting for the strong bioactivity of the Δ*lytB* mutant was characterised as a small pyrophosphorylated compound with a molecular mass of 262 Da, and purified by preparative anion exchange chromatography. Subsequently, the substance obtained was identified by electrospray-

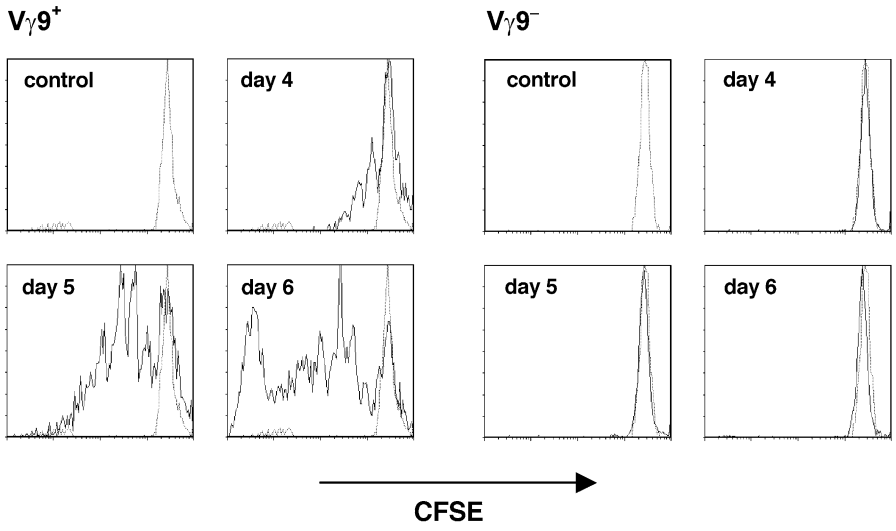

Fig. 2. Activation of human peripheral blood mononuclear cells by HMB-PP. Human peripheral blood mononuclear cells were labelled with 5-(and 6-)carboxyfluorescein diacetate succinimidyl ester (CFSE) and incubated with 0.25 nM HMB-PP in the presence of 100 U/ml interleukin-2 [45]. After 4, 5, and 6 days, cells were stained with CD3-phycoerythrin-Texas Red and Vγ9-phycoerythrin-cyanin 5.1 monoclonal antibodies, and dilution of the CFSE signal was measured in the $V\gamma9^+$ $CD3^+$ (= Vγ9/Vδ2 T cells) and $V\gamma9^-$ $CD3^+$ gate (= mainly $CD4^+$ and $CD8^+$ αβ T cells). Control, cells incubated for six days in the absence of HMB-PP.

ionisation mass spectroscopy, ^1H, ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectroscopy, and NOESY (nuclear Overhauser effect spectroscopy) analysis as HMB-PP [35]. At that time, HMB-PP was a novel metabolite that had not been described before, and turned out to be approx. 10 000 times more potent in stimulating Vγ9/Vδ2 T cells than IPP. Although there was only little evidence that HMB-PP was a true intermediate of the MEP pathway and not merely a side-product, the chemical structure of HMB-PP was in accordance with incorporation studies using deuterium-labelled methyl erythritol isotopomers [36], and insinuated a plausible mechanism for the unclear biosynthetic reaction sequence from MEcPP to IPP. In earlier studies, a highly immunogenic molecule of 262 Da with a comparable bioactivity had already been isolated from different mycobacterial species [37,38], and later from *E. coli* [32]. However, the chemical structure described as 3-formyl-1-butyl pyrophosphate (FBPP, TUBag1) has to remain speculative as the NMR data available differed from those of related compounds [35]. The existence of FBPP was mainly deduced from mass spectrometry analyses that rather suggest that the compound purified was actually HMB-PP, which results in an identical fragmentation pattern.

Since its first isolation from Δ*lytB E. coli* cells, the chemical synthesis of HMB-PP has been achieved by a number of laboratories [33,39–44]. Importantly, synthetic HMB-PP and the natural compound isolated from *E. coli* Δ*lytB* mutants displayed identical activities in stimulating Vγ9/Vδ2 T cells [33–35]. The reactivity of human peripheral blood mononuclear cells towards HMB-PP was restricted to Vγ9/Vδ2 T cells, leading to up-regulation of activation markers on the cell surface, secretion of pro-inflammatory cytokines, and expansion of the Vγ9/Vδ2 subpopulation in the presence of co-stimulation provided by interleukin-2 [33–35,45] (Fig. 2). In long-term Vγ9/Vδ2 T cell lines, HMB-PP triggered sustained T cell receptor signalling (Lafont, V. and Eberl, M., unpublished). While the observed effects were in line with earlier investigations on the weak agonist IPP, HMB-PP had an EC_{50} value of approx. 0.1 nM (compared to IPP with an EC_{50} of approx. 1 μM) [33], making HMB-PP by far the most potent and specific activator known for Vγ9/Vδ2 T cells [46]. Thus, it is likely that in fact HMB-PP exclusively accounts for the well-described Vγ9/Vδ2 T cell reactivity towards pathogenic bacteria such as *Brucella*, *Campylobacter*, *Ehrlichia*, *E. coli*, *Francisella*, *Listeria*, *Mycobacterium*, *Pseudomonas*, *Salmonella*, and *Yersinia*, as well as to the protozoan parasites *Plasmodium* and *Toxoplasma* (reviewed in [47]), in all of which the genes of the MEP pathway can be identified (Table 1).

4. Verification of HMB-PP as the missing link in the MEP pathway

As Δ*gcpE* mutants did not display any Vγ9/Vδ2 T cell bioactivity, GcpE was deduced to come first within the reaction sequence of the MEP pathway, before LytB [29,34]. Moreover, the fact that Δ*gcpE* and Δ*lytB E. coli* mutants accumulated MEcPP and HMB-PP, respectively, suggested these metabolites to represent the putative substrates of the corresponding enzymes [29,34,48]. Also, production of a phosphorylated derivative of (*E*)-2-methylbut-2-ene-1,4-diol (most likely representing HMB-PP) from radioactively labelled MEcPP was observed in crude extracts of *E. coli* cells over-expressing the three genes *yfgA*, *yfgB* and *gcpE*, which appear in a single gene cluster on the *E. coli* chromosome [49]. In analogous studies, in *E. coli* cells over-expressing all genes of the MEP pathway from *dxs* down to *gcpE* on a single artificial operon, enrichment of HMB-PP could be detected in the bacterial lysate [50]. Similarly, the production of IPP and DMAPP was observed when in addition the operon also included the *lytB* gene [51], indicating that HMB-PP indeed represented the long-sought missing link in the reaction sequence between MEcPP and IPP.

Eventually, an active recombinant GcpE from the hyperthermophilic bacterium *Thermus thermophilus* could be successfully expressed in *E. coli* and purified under oxygen-free

Table 1
The MEP pathway in human pathogenic, opportunistic, and commensal microbes

Species	*dxs*	*dxr*	*ygbP*	*ychB*	*ygbB*	*gcpE*	*lytB*
Acinetobacter sp.	?	?	?	?	?	?	+
Actinobacillus pleuropneumoniae[b]	+	+	+	+	+	+	+
Actinobacillus actinomycetemcomitans[b]	+	+	+	+	+	+	+
Anaplasma phagocytophilum[b]	+	+	+	+	+	+	+
Bacillus anthracis[a]	+	+	+	+	+	+	+
Bacillus cereus[b]	+	+	+	+	+	+	+
Bacteroides fragilis[b]	+	+	+	?	+	+	+
Bacteroides thetaiotaomicron[a]	+	+	+	+	+	+	+
Bifidobacterium longum[b]	+	+	+	+	+	+	+
Bordetella bronchiseptica[b]	+	+	+	+	+	+	+
Bordetella pertussis[b]	+	+	+	+	+	+	+
Brucella melitensis[a]	+	+	+	+	+	+	+
Brucella suis[a]	+	?	+	+	+	+	+
Burkholderia cepacia[b]	+	+	+	+	+	+	+
Burkholderia mallei[b]	+	+	+	+	+	+	+
Burkholderia pseudomallei[b]	+	+	+	+	+	+	+
Campylobacter jejuni[a]	+	+	+	+	+	+	+
Chlamydia trachomatis[a]	+	+	+	+	+	+	+
Chlamydophila pneumoniae[a]	+	+	+	+	+	+	+
Clostridium botulinum[b]	+	+	+	+	+	+	+
Clostridium perfringens[a]	+	+	+	+	+	+	+
Clostridium difficile[b]	+	+	+	+	+	+	+
Clostridium tetani[a]	+	+	+	+	+	+	+
Corynebacterium diphtheriae[b]	+	+	+	+	+	+	+
Ehrlichia chaffeensis[b]	+	+	+	+	+	+	+
Escherichia coli[a]	+	+	+	+	+	+	+
Eubacterium sp.	?	?	?	?	?	?	?
Francisella tularensis[b]	+	+	+	+	+	+	+
Fusobacterium nucleatum[b]	+	+	+	+	+	+	+
Gardnerella vaginalis	?	?	?	?	?	?	?
Haemophilus ducreyi[b]	+	+	+	+	+	+	+
Haemophilus influenzae[a]	+	+	+	+	+	+	+
Helicobacter pylori[a]	+	+	+	+	+	+	+
Klebsiella pneumoniae[b]	+	+	+	?	+	+	?
Leptospira interrogans[a]	+	+	+	+	+	+	+
Listeria monocytogenes[a]	+	+	+	+	+	+	+
Mannheimia haemolytica[b]	+	+	+	+	+	+	+
Moraxella catarrhalis[a]	+	+	+	+	+	+	+
Mycobacterium leprae[a]	+	+	+	+	+	+	+
Mycobacterium smegmatis[b]	+	+	+	+	+	+	+
Mycobacterium tuberculosis[a]	+	+	+	+	+	+	+
Mycoplasma penetrans[b]	+	+	+	?	+	+	+
Neisseria gonorrhoeae[b]	+	+	+	+	+	+	+
Neisseria meningitidis[a]	+	+	+	+	+	+	+
Neorickettsia sennetsu[b]	+	+	+	+	+	+	+
Pasteurella multocida[a]	+	+	+	+	+	+	+
Peptostreptococcus sp.	?	?	?	?	?	?	?
Porphyromonas gingivalis[b]	+	+	+	+	+	+	+
Prevotella intermedia[b]	+	+	+	+	+	+	+
Proteus mirabilis	?	?	?	?	?	?	?
Providencia stuartii	?	?	?	?	?	+	?
Pseudomonas aeruginosa[a]	+	+	+	+	+	+	+
Pseudomonas putida[a]	+	+	+	+	+	+	+
Psychrobacter sp.[b]	+	+	?	+	+	+	+
Salmonella enterica[a]	+	+	+	+	+	+	+
Salmonella enteritidis[b]	+	+	+	+	+	+	+
Salmonella typhimurium[a]	+	+	+	+	+	+	+
Serratia marcescens[b]	+	+	+	+	+	+	+
Shewanella putrefaciens[b]	+	+	+	+	+	+	+
Shigella flexneri[a]	+	+	+	+	+	+	+
Shigella dysenteriae[b]	+	+	+	+	+	+	+
Tannerella forsythensis[b]	+	+	+	+	+	+	+
Treponema denticola[b]	+	+	+	+	+	+	+
Treponema pallidum[a]	+	+	+	+	+	+	+
Tropheryma whipplei[a]	+	+	+	+	+	+	+
Vibrio cholerae[a]	+	+	+	+	+	+	+
Vibrio vulnificus[a]	+	+	+	+	+	+	+
Wolbachia sp.[b]	+	+	+	+	+	+	+
Yersinia pestis[a]	+	+	+	+	+	+	+
Yersinia enterocolitica[b]	+	+	+	+	+	+	+

Table 1 (*Continued*).

Species	*dxs*	*dxr*	*ygbP*	*ychB*	*ygbB*	*gcpE*	*lytB*
Cryptosporidium parvum	+	?	+	?	+	+	?
Plasmodium falciparum[a]	+	+	+	+	+	+	+
Plasmodium vivax[b]	+	+	+	+	+	+	+
Toxoplasma gondii	+	+	?	?	+	+	+

Homologues to *E. coli* genes were identified by TBLASTN searches at www.ncbi.nlm.nih.gov, www.tigr.org, www.jgi.doe.gov, www.parvum.mic.vcu.edu, www.genedb.org, hgsc.bcm.tmc.edu, and artedi.ebc.uu.se. In some species (e.g. *B. suis*, *B. melitensis*, *C. jejuni*, *H. pylori*, *T. pallidum*, *T. whipplei*, and others), *ygbP* and *ygbB* are within the same open reading frame and encode a YgbP–YgbB fusion protein. *M. penetrans* is the only *Mycoplasma* species sequenced so far having the MEP pathway, which is absent in *M. genitalium*, *M. pneumoniae*, and *M. pulmonis*. *L. monocytogenes* harbours both the MEP and the mevalonate pathway.
[a]Complete genome available.
[b]Whole genome shotgun sequence available.

conditions. In an in vitro assay, this protein was enzymatically active and converted MEcPP into HMB-PP in the presence of dithionite as reducing agent [52]. Other investigators expressed a fusion protein encompassing the *E. coli* GcpE and a maltose-binding domain, and demonstrated that, whereas the purified protein was inactive, the described activity could be restored by addition of a crude cell extract from a Δ*gcpE* mutant, suggesting the requirement of auxiliary factors present in bacterial extract [53]. Similarly, while a fusion protein of the *E. coli* LytB protein and the maltose-binding domain was unable to catalyse the in vitro transformation of HMB-PP, the recombinant protein increased the low intrinsic catalytic activity of *E. coli* wild-type extract [54]. During the course of these studies, HMB-PP was shown by in vitro and in vivo experiments to serve as the biosynthetic precursor of both IPP and DMAPP, which were obtained in a ratio of 4:1–6:1 by the catalytic action of LytB [51,53,54]. The apparent formation of IPP and DMAPP by a single enzyme was in remarkable contrast to the mevalonate pathway where DMAPP is successively synthesised from IPP by IPP isomerase. Yet, branching of the MEP pathway for the formation of IPP and DMAPP had already been suggested earlier based on isotope-labelling experiments and the fact that *E. coli* deletion mutants for IPP isomerase were still viable, while a *lytB* deficiency was lethal [24–26,55]; even more, many bacteria with the MEP pathway do not possess IPP isomerases [12]. In a defined in vitro assay under exclusion of oxygen, incubation of recombinant LytB from the thermophilic bacterium *Aquifex aeolicus* with HMB-PP and dithionite as artificial electron donor resulted in the formation of IPP and DMAPP at a ratio of approx. 5:1 [56].

Only now can be appreciated why the investigation of the terminal reaction steps has been hampered for so long. Both GcpE and LytB contain [4Fe–4S] clusters and are therefore highly oxygen sensitive, which had already been predicted from the existence of three conserved cysteine residues [43]. The presence of these clusters was evidenced by the observation that recombinant GcpE and LytB displayed typical absorptions at 413–420 nm in their UV/visible spectra [52,53,56], and by data obtained by electron paramagnetic resonance spectroscopy (Duin, E.C., Altincicek, B. and Kollas, A.K., unpublished). Additional support for such a prosthetic group was provided by the successful in vitro reconstitution of the [4Fe–4S] cluster of *E. coli* GcpE and its enzymatic activity using photoreduced 5-deazaflavin or a flavodoxin/flavodoxin reductase regeneration system [57]. Although the natural cofactors and electron donors for GcpE and LytB still need to be found, the main steps of the MEP pathway can thus be considered cracked.

5. Conclusions

With the enzymes and intermediates of the MEP pathway being identified, it is becoming increasingly clear that this reaction cascade is more than merely an alternative pathway of isoprenoid biosynthesis. Apart from its relevance for isoprenoid biosynthesis, some intermediates of the pathway can also serve as substrates for other pathways branching off the isoprenoid mainline. While DOXP is also a precursor of thiamine and pyridoxal, it has recently been shown that HMB-PP is used for the synthesis of the plant hormone zeatin by *Agrobacterium tumefaciens* [58]. In contrast, the physiological role for the apparent occurrence of uridine- and thymidine-conjugates of HMB-PP in mycobacteria is not clear yet [38,59]. Whether HMB-PP is involved in further biosynthetic functions and whether other metabolites also play additional roles remains to be clarified. Still, the involvement of the MEP pathway in providing the Vγ9/Vδ2 T cell activator is the most intriguing outcome of these biochemical studies.

The highly specific reactivity of Vγ9/Vδ2 T cells has now been resolved, but its biological implication still poses a number of questions. Vγ9/Vδ2 T cells have manifold functions and exhibit the classical type 1/type 2 cytokine dichotomy already established for CD4 and CD8 T cells [60]. Also, the existence of naive, effector memory, and central memory Vγ9/Vδ2 T cells parallels findings on CD4 and CD8 T cells [45]. But what is the precise role of these multipotent T cell populations? And why does the efficient recognition of low molecular weight compounds not exist in other mammalians, with γδ T cells being supposedly the first step in the evolution of adaptive immunity more than 400 million years ago [61]? Some authors suggested that a major function of Vγ9/Vδ2 T cells might be scrutinising the body for transformed cells that are metabolically altered and display an imbalanced IPP biosynthesis. Thus, damaged or necrotic cells might release IPP or higher isoprenoid units like farnesyl-PP or geranyl-PP during the course of viral, bacterial or parasitic infections [62]. Also, the (hypothetical) accumulation of these relatively poor ligands might represent some kind of 'danger' signal for the immune system [63] (although the inhibitory effect of mevastatin and other HMG-CoA reductase blockers on Vγ9/Vδ2 T cell activation might be mediated through influencing post-translational isoprenylation of proteins involved in cell division and maturation, rather than IPP depletion [64]). At the same time it is thinkable that recognition of HMB-PP and related compounds by Vγ9/Vδ2 T cells is a mechanism developed during primate evolution to allow a specific but broad reactivity towards many microbial pathogens, by targeting a distinctive and vital metabolic route shared by these organ-

isms – most human pathogens possess the MEP pathway (with *Streptococcus*, *Staphylococcus* and *Borrelia* being the main exceptions). Due to this unconventional recognition pattern, Vγ9/Vδ2 T cells would not need to traffic through lymphoid organs where only rare antigens have to be processed and presented to highly specific cognate antigen receptors [65]. Still, if Vγ9/Vδ2 T cells evolved to detect both stressed cells and microbial pathogens, this could only be achieved if the local amounts of IPP released from damaged tissues were some 10 000-fold higher than the HMB-PP concentrations reached during bacterial infections, to allow for the hugely different bioactivities of these two compounds.

In many cases though, the rapid and strong reactivity of Vγ9/Vδ2 T cells towards HMB-PP seems somewhat ineffective, as many bacterial species manage to escape the immune response nonetheless and establish chronic and debilitating infections – among these are some of the oldest and severest infectious diseases of humans, such as tuberculosis, leprosy, syphilis, and malaria. Even more, why have these pathogens not succeeded in avoiding the suicidal presentation of such a highly immunogenic compound to the host's immune system? Importantly, not only do many pathogenic and opportunistic bacteria use the MEP pathway, but also the most frequent bacteria species in the enteric flora and human faeces, *Bacteroides*, *Bifidobacterium*, *Fusobacterium*, *E. coli*, and *Clostridium* (and probably also *Eubacterium* and *Peptostreptococcus*). Thus, Vγ9/Vδ2 T cells are more likely to play a role in maintaining the physiological function of the intestine and might primarily have evolved to initiate and regulate the mucosal immune system in order to limit a detrimental host response towards commensal bacteria. If this is the case, do pathogens then deliberately exploit this surveillance system during infiltration of the body, thereby inducing a state of tolerance? Basically, it still remains puzzling how a molecule as simple as HMB-PP can be recognised with such a high specificity by the Vγ9/Vδ2 T cell receptor, and how – if at all – the host benefits from this property in microbial infection.

Acknowledgements: The authors have received support by grants from the Bundesministerium für Bildung und Forschung (BioChance 0312588), the Deutsche Forschungsgemeinschaft (SPP1131), the Fonds der Chemischen Industrie (SK 172/5), and the European Commission (ICA4-2000-10290 and QLRT-2001-00887).

References

[1] Kabelitz, D., Bender, A., Schondelmaier, S., Schoel, B. and Kaufmann, S.H. (1990) J. Exp. Med. 171, 667–679.
[2] Goerlich, R., Hacker, G., Pfeffer, K., Heeg, K. and Wagner, H. (1991) Eur. J. Immunol. 21, 2613–2616.
[3] Pfeffer, K., Schoel, B., Plesnila, N., Lipford, G.B., Kromer, S., Deusch, K. and Wagner, H. (1992) J. Immunol. 148, 575–583.
[4] Constant, P., Davodeau, F., Peyrat, M.A., Poquet, Y., Puzo, G., Bonneville, M. and Fournié, J.J. (1994) Science 264, 267–270.
[5] Banthorpe, D.V., Charlwood, B.V. and Francis, M.J. (1972) Chem. Rev. 72, 115–155.
[6] Rohmer, M., Knani, M., Simonin, P., Sutter, B. and Sahm, H. (1993) Biochem. J. 295, 517–524.
[7] Schwarz, M.K. (1994) Ph.D. Thesis No. 10951, ETH Zürich, Switzerland.
[8] Broers, S.T.J. (1994) Ph.D. Thesis No. 10978, ETH Zürich, Switzerland.
[9] Arigoni, D., Sagner, S., Latzel, C., Eisenreich, W., Bacher, A. and Zenk, M.H. (1997) Proc. Natl. Acad. Sci. USA 94, 10600–10605.
[10] Rohmer, M. (1999) Nat. Prod. Rep. 16, 565–574.
[11] Lichtenthaler, H.K. (2000) Biochem. Soc. Trans. 28, 785–789.
[12] Rohdich, F., Kis, K., Bacher, A. and Eisenreich, W. (2001) Curr. Opin. Chem. Biol. 5, 535–540.
[13] Jomaa, H., Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Turbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H.K., Soldati, D. and Beck, E. (1999) Science 285, 1573–1576.
[14] Sprenger, G.A., Schörken, U., Wiegert, T., Grolle, S., de Graaf, A.A., Taylor, S.V., Begley, T.P., Bringer-Meyer, S. and Sahm, H. (1997) Proc. Natl. Acad. Sci. USA 94, 12857–12862.
[15] Lois, L.M., Campos, N., Putra, S.R., Danielsen, K., Rohmer, M. and Boronat, A. (1998) Proc. Natl. Acad. Sci. USA 95, 2105–2110.
[16] Takahashi, S., Kuzuyama, T. and Watanabe, H. (1998) Proc. Natl. Acad. Sci. USA 95, 9879–9884.
[17] Lange, B.M. and Croteau, R. (1999) Arch. Biochem. Biophys. 365, 170–174.
[18] Schwender, J., Müller, C., Zeidler, J. and Lichtenthaler, H.K. (1999) FEBS Lett. 455, 140–144.
[19] Rohdich, F., Wungsintaweekul, J., Fellermeier, M., Sagner, S., Herz, S., Kis, K., Eisenreich, W., Bacher, A. and Zenk, M.H. (1999) Proc. Natl. Acad. Sci. USA 96, 11758–11763.
[20] Lüttgen, H., Rohdich, F., Herz, S., Wungsintaweekul, J., Hecht, S., Schuhr, C.A., Fellermeier, M., Sagner, S., Zenk, M.H., Bacher, A. and Eisenreich, W. (2000) Proc. Natl. Acad. Sci. USA 97, 1062–1067.
[21] Herz, S., Wungsintaweekul, J., Schuhr, C.A., Hecht, S., Lüttgen, H., Sagner, S., Fellermeier, M., Eisenreich, W., Zenk, M.H., Bacher, A. and Rohdich, F. (2000) Proc. Natl. Acad. Sci. USA 97, 2486–2490.
[22] Altincicek, B., Kollas, A.K., Sanderbrand, S., Wiesner, J., Hintz, M., Beck, E. and Jomaa, H. (2001) J. Bacteriol. 183, 2411–2416.
[23] Campos, N., Rodríguez-Concepción, M., Seemann, M., Rohmer, M. and Boronat, A. (2001) FEBS Lett. 488, 170–173.
[24] Altincicek, B., Kollas, A.K., Eberl, M., Wiesner, J., Sanderbrand, S., Hintz, M., Beck, E. and Jomaa, H. (2001) FEBS Lett. 499, 37–40.
[25] Cunningham Jr., F.X., Lafond, T.P. and Gantt, E. (2000) J. Bacteriol. 182, 5841–5848.
[26] McAteer, S., Coulson, A., McLennan, N. and Masters, M. (2001) J. Bacteriol. 183, 7403–7407.
[27] Tanaka, Y., Morita, C.T., Tanaka, Y., Nieves, E., Brenner, M.B. and Bloom, B.R. (1995) Nature 375, 155–158.
[28] Jomaa, H., Feurle, J., Luhs, K., Kunzmann, V., Tony, H.P., Herderich, M. and Wilhelm, M. (1999) FEMS Immunol. Med. Microbiol. 25, 371–378.
[29] Altincicek, B., Moll, J., Campos, N., Foerster, G., Beck, E., Hoeffler, J.F., Grosdemange-Billiard, C., Rodríguez-Concepción, M., Rohmer, M., Boronat, A., Eberl, M. and Jomaa, H. (2001) J. Immunol. 166, 3651–3654.
[30] Kuzuyama, T., Takahashi, S. and Seto, H. (1999) Biosci. Biotechnol. Biochem. 63, 776–778.
[31] Potapov, V.D., Biketov, S.F., Demina, G.R., Lysak, E.I., Titareva, G.M., Bakhteeva, I.V. and Ostrovsky, D.N. (2001) Appl. Biochem. Microbiol. 37, 238–241.
[32] Feurle, J., Espinosa, E., Eckstein, S., Pont, F., Kunzmann, V., Fournié, J.J., Herderich, M. and Wilhelm, M. (2002) J. Biol. Chem. 277, 148–154.
[33] Reichenberg, A., Hintz, M., Kletschek, Y., Kuhl, T., Haug, C., Engel, R., Moll, J., Ostrovsky, D.N., Jomaa, H. and Eberl, M. (2003) Bioorg. Med. Chem. Lett. 13, 1257–1260.
[34] Eberl, M., Altincicek, B., Kollas, A.K., Sanderbrand, S., Bahr, U., Reichenberg, A., Beck, E., Foster, D., Wiesner, J., Hintz, M. and Jomaa, H. (2002) Immunology 106, 200–211.
[35] Hintz, M., Reichenberg, A., Altincicek, B., Bahr, U., Gschwind, R.M., Kollas, A.K., Beck, E., Wiesner, J., Eberl, M. and Jomaa, H. (2001) FEBS Lett. 509, 317–322.
[36] Charon, L., Hoeffler, J.F., Pale-Grosdemange, C., Lois, L.M., Campos, N., Boronat, A. and Rohmer, M. (2000) Biochem. J. 346, 737–742.
[37] De Libero, G. (1997) Immunol. Today 18, 22–26.
[38] Belmant, C., Espinosa, E., Poupot, R., Peyrat, M.A., Guiraud, M., Poquet, Y., Bonneville, M. and Fournié, J.J. (1999) J. Biol. Chem. 274, 32079–32084.
[39] Fox, D.T. and Poulter, C.D. (2002) J. Org. Chem. 67, 5009–5010.

[40] Ward, J.L. and Beale, M.H. (2002) J. Chem. Soc. Perkin Trans. 1 6, 710–712.
[41] Gao, W., Loeser, R., Raschke, M., Dessoy, M.A., Fulhorst, M., Alpermann, H., Wessjohann, L.A. and Zenk, M.H. (2002) Angew. Chem. Int. Ed. Engl. 41, 2604–2607.
[42] Giner, J.L. (2002) Tetrahedron Lett. 43, 5457–5459.
[43] Wolff, M., Seemann, M., Grosdemange-Billiard, C., Tritsch, D., Campos, N., Rodríguez-Concepción, M., Boronat, A. and Rohmer, M. (2002) Tetrahedron Lett. 43, 2555–2559.
[44] Amslinger, S., Kis, K., Hecht, S., Adam, P., Rohdich, F., Arigoni, D., Bacher, A. and Eisenreich, W. (2002) J. Org. Chem. 67, 4590–4594.
[45] Eberl, M., Engel, R., Beck, E. and Jomaa, H. (2002) Cell. Immunol. 218, 1–6.
[46] Gossman, W. and Oldfield, E. (2002) J. Med. Chem. 45, 4868–4874.
[47] Sicard, H. and Fournié, J.J. (2000) Infect. Immun. 68, 4375–4377.
[48] Seemann, M., Campos, N., Rodríguez-Concepción, M., Hoeffler, J.F., Grosdemange-Billiard, C., Boronat, A. and Rohmer, M. (2002) Tetrahedron Lett. 43, 775–778.
[49] Seemann, M., Campos, N., Rodríguez-Concepción, M., Ibañez, E., Duvold, T., Tritsch, D., Boronat, A. and Rohmer, M. (2002) Tetrahedron Lett. 43, 1413–1415.
[50] Hecht, S., Eisenreich, W., Adam, P., Amslinger, S., Kis, K., Bacher, A., Arigoni, D. and Rohdich, F. (2001) Proc. Natl. Acad. Sci. USA 98, 14837–14842.
[51] Rohdich, F., Hecht, S., Gartner, K., Adam, P., Krieger, C., Amslinger, S., Arigoni, D., Bacher, A. and Eisenreich, W. (2002) Proc. Natl. Acad. Sci. USA 99, 1158–1163.
[52] Kollas, A.K., Duin, E.C., Eberl, M., Altincicek, B., Hintz, M., Reichenberg, A., Henschker, D., Henne, A., Steinbrecher, I., Ostrovsky, D.N., Hedderich, R., Beck, E., Jomaa, H. and Wiesner, J. (2002) FEBS Lett. 532, 432–436.
[53] Rohdich, F., Zepeck, F., Adam, P., Hecht, S., Kaiser, J., Laupitz, R., Grawert, T., Amslinger, S., Eisenreich, W., Bacher, A. and Arigoni, D. (2003) Proc. Natl. Acad. Sci. USA 100, 1586–1591.
[54] Adam, P., Hecht, S., Eisenreich, W., Kaiser, J., Grawert, T., Arigoni, D., Bacher, A. and Rohdich, F. (2002) Proc. Natl. Acad. Sci. USA 99, 12108–12113.
[55] Rodríguez-Concepción, M., Campos, N., Maria Lois, L., Maldonado, C., Hoeffler, J.F., Grosdemange-Billiard, C., Rohmer, M. and Boronat, A. (2000) FEBS Lett. 473, 328–332.
[56] Altincicek, B., Duin, E.C., Reichenberg, A., Hedderich, R., Kollas, A.K., Hintz, M., Wagner, S., Wiesner, J., Beck, E. and Jomaa, H. (2002) FEBS Lett. 532, 437–440.
[57] Seemann, M., Bui, B.T., Wolff, M., Tritsch, D., Campos, N., Boronat, A., Marquet, A. and Rohmer, M. (2002) Angew. Chem. Int. Ed. Engl. 41, 4337–4339.
[58] Krall, L., Raschke, M., Zenk, M. and Baron, C. (2002) FEBS Lett. 527, 315–318.
[59] Poquet, Y., Constant, P., Halary, F., Peyrat, M.A., Gilleron, M., Davodeau, F., Bonneville, M. and Fournié, J.J. (1996) Eur. J. Immunol. 26, 2344–2349.
[60] Wesch, D., Glatzel, A. and Kabelitz, D. (2001) Cell. Immunol. 212, 110–117.
[61] Matsunaga, T. and Rahman, A. (1998) Immunol. Rev. 166, 177–186.
[62] De Libero, G. (1997) Immunol. Today 18, 22–26.
[63] Gober, H.J., Kistowska, M., Angman, L., Jeno, P., Mori, L. and De Libero, G. (2003) J. Exp. Med. 197, 163–168.
[64] Youssef, S., Stuve, O., Patarroyo, J.C., Ruiz, P.J., Radosevich, J.L., Hur, E.M., Bravo, M., Mitchell, D.J., Sobel, R.A., Steinman, L. and Zamvil, S.S. (2002) Nature 420, 78–84.
[65] Hayday, A.C. and Tigelaar, R. (2003) Nat. Rev. Immunol. 3, 233–242.